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Allocation to highly sensitized patients based on acceptable mismatches results in low rejection rates comparable to non-sensitized patients

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Abbreviations:

AM: acceptable mismatch

HB: heart beating

HLA: human leucocyte antigen

CDC: complement dependent cytotoxicity

DGF: delayed graft function transplant through the AM program

DSA: donor specific antibody

ENIS: Eurotransplant Network Information System

ETKAS: Eurotransplant kidney allocation system

IL2RA: IL-2 receptor antagonist

MMF: mycophenolate mofetil

NHB: non-heart beating

NIMA: noninherited maternal antigen

PBMC: peripheral blood mononuclear cell

PRA: panel reactive antibody

SAB: single antigen bead

Abstract

Whereas regular allocation avoids unacceptable mismatches on the donor organ, allocation to highly sensitized patients within the Eurotransplant Acceptable Mismatch (AM) program is based on the patient's HLA phenotype plus acceptable antigens. These are HLA antigens to which the patient never made antibodies, determined by extensive laboratory testing. AM patients have superior long-term graft survival compared to highly sensitized patients in regular allocation. Here, we questioned whether the AM program also results in lower rejection rates. From the PROCARE cohort, consisting of all Dutch kidney transplants 1995-2005, we selected deceased donor single transplants with minimum one HLA mismatch and determined the cumulative 6-month rejection incidence for patients in AM or regular allocation. Additionally, we determined the effect of minimal matching criteria of one HLA-B plus one HLA-DR, or two HLA-DR antigens on rejection incidence. AM patients showed significantly lower rejection rates than highly immunized patients in regular allocation, comparable to non-sensitized patients, independent of other risk factors for rejection. Contrasting to highly sensitized patients in regular allocation, minimal matching criteria did not affect rejection rates in AM patients. Allocation based on acceptable antigens leads to relatively low risk transplants for highly sensitized patients with rejection rates similar to non-immunized individuals.

Introduction

Sensitization towards Human Leucocyte Antigens (HLA) can occur through pregnancy, blood transfusion, or transplantation (1). When a patient has formed antibodies reactive >85% of HLA antigens present in the donor population, this patient is regarded as being highly sensitized (2). Highly sensitized patients accrue on the transplant waiting list due to the low number of available crossmatch negative donors. The Eurotransplant Acceptable Mismatch program was established almost 30 years ago with the aim to provide a chance for highly sensitized patients to be transplanted, and has resulted in more than 1500 transplants (3). The program is based on the positive identification of HLA antigens to which the patient has not made any antibodies by using extensive laboratory testing (4). Acceptable antigens are added to the HLA phenotype of the patient, creating an 'extended' HLA

phenotype, which is used for allocation (5). Any available deceased donor organ that matches this extended phenotype is mandatorily shipped to the AM patient, resulting in lower waiting times for these highly sensitized patients (6, 7). Acceptable antigens are truly acceptable, since no HLA match effect is observed in patients transplanted through the AM program (5, 7). Previously, it was shown that the long-term graft survival of patients transplanted through the AM program is far superior to that of their highly sensitized counterparts transplanted through regular allocation, and was even comparable to non-sensitized patients (7, 8). Since the AM strategy is targeted at defining HLA antigens that are immunologically acceptable it is to be expected that allocation based on acceptable antigens would also result in a lower rejection incidence. Unfortunately, due to lack of registration of rejection data in the Eurotransplant Network Information System (ENIS), it has not been possible so far to determine the effect of the AM approach on rejection rates. The Dutch multi-center PROCARE study, which includes clinical follow-up of all kidney transplants performed between 1995 and 2005 in the Netherlands, allowed for the first time to determine the effect of allocation based on acceptable mismatches on rejection rates.

Methods

The AM program

Current eligibility criteria for inclusion into the AM program are a cumulative waiting time on the Eurotransplant Kidney Allocation System (ETKAS) waiting list of at least 2 years, and a complement-dependent cytotoxicity (CDC) PRA of >85% in either historic or current serum samples. In the time period 1995-2005 acceptable antigens were defined by making use of mainly cellular assays as described elsewhere (5). Briefly, CDC assays were performed using patient specific cell panels of lymphocytes that had only one HLA mismatch with the patient, in which negative reactions would specify acceptable antigens. Similarly, a panel of K562 cells lines transfected with genes encoding single HLA class I alleles were used as targets in CDC. In the time period studied, solid phase assays were not routinely used.

For allocation purposes, HLA matching on the patient's own HLA antigens and additional acceptable antigens were performed on the split antigen level. Minimal match criteria on identity of either two HLA-DR antigens or one HLA-DR antigen with one HLA-B antigen at the split level were adhered to. For patients with a chance of receiving a kidney through the AM program of <0.1% (based on immunological grounds), minimal HLA matching was reduced to one HLA-DR match with the patient on the broad antigen level. Furthermore, whereas regular allocation through ETKAS is based on blood group identity, AM patients are transplanted based on blood group compatibility.

Patients

We performed a *post hoc* analysis on the PROCARE cohort, which includes all renal transplantations performed in the Netherlands between January 1995 and December 2005 with available clinical follow-up (9). All transplantations required a negative CDC crossmatch using both peak and current sera. A detailed description of the cohort has been published previously (10). Clinical data were obtained from the Dutch Organ Transplant Registry. Rejection was defined as the presence of biopsy-proved acute rejection (without further classification), or any treatment for acute rejection when no biopsy was performed. Patients transplanted through regular allocation (ETKAS) were grouped according to the level of sensitization (0%–5% peak PRA: non-sensitized; 6%–85% peak PRA: intermediately sensitized; and >85% peak PRA: highly sensitized), as defined by CDC assays. Patients included on the AM waiting list remained on the ETKAS waiting list as well, and those actually transplanted through ETKAS (and thus received an organ based on the absence of unacceptable antigens only) are included in the >85% PRA ETKAS group. The study design is schematically depicted in supplemental Figure S1, and patient characteristics are depicted in table 1. All patients provided written informed consent for use of their clinical data. The study protocol was approved by the Biobank Research Ethics Committee of the UMC Utrecht (TC Bio 13-633) and performed in accordance with the Declaration of Helsinki.

Detection and definition of DSAs by solid phase

All available pre-transplant patient sera were retrospectively tested for the presence of donor specific antibodies (DSA) by luminex single antigen bead (SAB) assays and analyzed in context of the PROCARE study as described previously (10).

Data handling

Groupings of quantitative variables were based on the following strategies: transplant period was divided into 2 equal periods, recipient and donor age of 50 years were used for stratification based on previous studies (11). Donor type was defined as either heart beating (HB) or non-heart beating (NHB). Initial immunosuppression was categorized as prednisolone / cyclosporin ± MMF ± IL-2 receptor antagonist (IL2RA) versus prednisolone / tacrolimus / MMF ± IL2RA based on a previous study on the complete PROCARE cohort (12). Graft function was categorized on direct or delayed function and HLA mismatches were divided into equal categories.

Statistical analysis

The Chi-squared test was used to test whether there is a trend in the proportions with transplant characteristics over the four categories. Statistical significance was determined by using the log-rank test, corrected for multiple comparisons (Bonferroni method), where applicable. Inclusion criterion for the multivariate analysis was a univariate *P*-value of <0.1. Multivariate Cox regression analysis was performed to determine independent effects on 6-month cumulative rejection incidence. *P*-values were 2-tailed, and those <0.05 were considered statistically significant. SPSS version 23 (IBM, Armonk, NY) and GraphPad Prism, version 7.04 (GraphPad Software, La Jolla, CA) were used.

Results

Allocation based on acceptable mismatches results in low rejection rates

To determine the effect of allocation based on acceptable mismatches on the 6-month cumulative rejection incidence, we selected all deceased donor single renal transplants from 1996 to 2005 (in 1996, ETKAS was initiated (13)) with at least one HLA antigen mismatch (HLA-A, -B or -DR) at the broad antigen level. We observed an increased rejection incidence with increased sensitization grade within regular allocation, with the highest incidence of rejection in the highly sensitized patients transplanted through ETKAS (Figure 1A). In contrast, highly sensitized patients transplanted through the AM program showed similar rejection rates to non-sensitized patients (P=1.000), and lower, although not significant, rejection rates as intermediately sensitized patients (P=0.423). When compared to their highly sensitized counterparts transplanted through regular allocation, AM patients experienced a significantly lower rejection incidence (P=0.004, Figure 1A). To determine the effect of the different allocation schemes on rejection rates later after transplant, we also analyzed the cumulative rejection incidence between 6 months and 5 years and observed no differences in this later period (Figure 1B).

We next performed univariate Cox regression analysis on all highly sensitized patients (n=234) with variables that potentially affect the rejection incidence (Table 2). The variables sex and age of recipient and donor, donor type, first transplant versus repeat transplant, HLA mismatch grade, transplant period, presence of SAB detected DSA class I, class II or both class I and II, and initial immunosuppression did not significantly affect the cumulative 6-month rejection incidence. The only variables that affected the incidence of rejection were delayed graft function (HR: 1.94; 95% CI: 1.19 to 3.17; P=0.008) and receiving a transplant through the AM program (HR: 0.47; 95% CI: 0.29 to 0.76; P=0.002). The variables transplant period, initial immunosuppression, initial graft function and transplant through the AM program were selected for subsequent multivariate analysis to determine whether the effect of receiving a transplant through the AM program was independent. For initial immunosuppression, there were missing values for 88 patients (38%) due to heterogenous

immunosuppression protocols outside the two main immunosuppression categories. To exclude an interaction between initial immunosuppression and transplant through the AM program we first analysed these variables in a separate multivariate analysis and observed only a minimal effect of initial immunosuppression on the variable transplant through the AM program (HR changes from 0.47 to 0.54, Table 2). Subsequent multivariate analysis on transplant period, initial immunosuppression, initial graft function and transplant through the AM program showed that only delayed graft function (HR: 1.93; 95% CI: 1.16 to 3.19; *P*=0.011) and receiving a transplant through the AM program (HR: 0.57; 95% CI: 0.34 to 0.95; *P*=0.029) were independently associated with 6-month cumulative rejection incidence (Table 2).

Minimal match criteria do not result in lower rejection rates in AM patients

It has previously been shown that AM patients transplanted with a minimal match level of two HLA-DR antigens, or one HLA-DR and one HLA-B antigen have similar graft survival rates compared to AM patients without this minimal level of HLA matching, raising the possibility that the minimal match criteria for AM patients could be abandoned (3). Importantly, in the current cohort we were able to determine the effect of the minimal match criteria on rejection rates. For this analysis, we included also patients with zero HLA mismatches (Figure S1). We found that receiving a transplant without the aforementioned minimal match level, but a minimum match of one HLA-DR on the broad antigen level, significantly increased the 6-month cumulative rejection incidence in patients transplanted through ETKAS (*P*<0.0001, Figure 2A), whereas no effect was found in the AM cohort (*P*=0.700, Figure 2B). The data indicate that the minimal match criteria are not beneficial over one HLA-DR broad antigen match for patients transplanted through the AM program.

Discussion

It is known that transplantation to sensitized patients through regular allocation is associated with an elevated risk for graft rejection (14-16). The current study confirms these data, with the cumulative rejection incidence for highly sensitized patients transplanted through ETKAS being almost double that of non-sensitized ETKAS patients. In contrast, patients transplanted through the AM program showed significantly lower rejection rates compared to highly sensitized patients transplanted through regular allocation, and even had similar rejection rates as non-sensitized patients. Upon multivariate analysis, receiving a transplant through the AM program remained independently associated with low rejection rates in highly sensitized patients. The occurrence of rejection is known to be a risk factor for subsequent inferior long-term graft survival (17). Indeed, it has been described previously that graft survival in AM patients is far superior to that in highly sensitized patients transplanted through ETKAS and comparable to that in non-sensitized ETKAS patients (7). Limitations of the study are that it does not include information on whether the rejections were biopsy proven. In the PROCARE database rejection was defined as a registered treatment for rejection, of which 56.4% (n= 456) were accompanied by a documented biopsy taken a day prior, or at the day of initiation of anti-rejection treatment, a percentage that was evenly distributed between the different groups (P=0.122, supplemental Table 1). This is likely an underestimation due to incompleteness of the database for this field. To get a more stringent selection on the rejection events, we determine the 6-month cumulative rejection of highly sensitized patients (ETKAS and AM) without any rejection or who received a documented biopsy-informed anti-rejection treatment defined as described above, and again found that patients transplanted through AM had a significantly lower rejection incidence than their highly sensitized counterparts transplanted through ETKAS (supplemental Figure S2).

Secondly, we were unable to further differentiate in type of rejection, since a classification of rejection is not available from the Dutch Organ Transplant Registry, and cannot be obtained retrospectively due to the various changes in BANFF criteria over time. Finally, there are no data available regarding development of *de novo* donor-specific antibodies in the current cohort. With the

current study showing a marked benefit for AM patients, these parameters should be included in a consecutive study on a more recent cohort.

The finding that allocation based on acceptable antigens results in low rejection rates and excellent long-term graft survival can be explained in several ways. Firstly, the absence of particular HLA antibody specificities is actively determined for AM patients in both historic and current sera, in contrast to regular allocation in which unacceptable antigens are determined, and all other antigens are presumed acceptable. Secondly, there is evidence that acquired neonatal tolerance explains a proportion of acceptable antigens, since acceptable antigens often include the noninherited maternal antigens (NIMA) (18, 19). Thirdly, acceptable antigens could either harbor a low level of epitope mismatches with the patient, or the epitope mismatches that are present are of low immunogenicity (20). Preliminary data suggest the latter, since analysis for HLA class I shows similar levels of epitope mismatches for AM patients and patients transplanted through regular allocation, with no effect of the number of epitope mismatches on graft survival for AM patients (Heidt et al, manuscript in preparation).

Currently, acceptable antigens for HLA-DQA, HLA-DPA and HLA-DPB are not yet accounted for in the AM program, which leaves the possibility that rejection rates for AM patients could be even lower when these loci are also taken into consideration. Indeed, HLA-DQ seems to be the dominant target for HLA antibodies after transplantation (21, 22). Future analyses should show whether extension of acceptable mismatches to these additional loci will indeed lead to better outcome. Such analyses should preferentially be performed in the whole AM population, since in the current study only transplants performed in the Netherlands were included. However, the definition of acceptable antigens is done centrally at the Eurotransplant Reference Laboratory, using the same criteria for all patients within Eurotransplant. While confirmation of our results within the whole of Eurotransplant is desirable, we expect similar results to the current study.

Besides a previously described lack of effect of minimal match criteria on long-term graft survival in AM patients (3), we here show a lack of effect on rejection incidence as well, confirming that acceptable mismatches are truly acceptable. Together, these data strongly support downscaling the minimal match criteria for AM patients to one HLA-DR broad antigen match, which can result in around 200 additional transplants to highly sensitized patients through the AM program each year (3).

Timely transplantation of highly sensitized patients is of the utmost importance, but should be accompanied by low rejection rates and long-term graft survival to have a true impact on the waiting list of highly sensitized patients. We here show that transplantation of highly sensitized patients can be achieved with comparable rejection rates to non-sensitized patients, when acceptable mismatches are used in the allocation process.

Author contributions

Analyzed the data: SH-GH-MvdLvO-MW-FC

Contributed reagents/materials/analysis tools: EK-TK-BW-IJ-WA-AvdM-LH-MB-ES-CH-FvR-AvZ-MV-MLB-AD-LP-JM-NB-MS-JS-BH-AL-LB-CR-MT-JV-CV-LW-EvD-MG-MC-FvI-AN-NL-WS-KvdP-NvdW-ItB-FB-AH-PvdB-JdF-MB-SH-DR-FC-HO

Evaluated kidney transplant patients: LH-MCB-FvR-AvZ-MV-MS-JS-EvD-MG-MC-FvI-AN-KvdP-NvdW-ItB-FB-AH-PvdB-JdF-MB

Contributed to writing of the manuscript: SH-GH-MvdLvO-MW-FC-HO-FB-MB-BH-ItB-CV-LH

All authors reviewed and approved the final version of the manuscript.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation.*

Data Availability Statement

Data available on request due to privacy/ethical restrictions.

Figure legends

Figure 1. (A) Comparison of 6-month cumulative rejection incidence between patients transplanted through the acceptable mismatch (AM) program or through the Eurotransplant Kidney Allocation System (ETKAS). (B) Comparison of 5-year cumulative rejection incidence between patients transplanted through the AM program or through ETKAS, for which rejection incidence was set at zero on 6 months. The ETKAS patients are subdivided based on their sensitization grade: 0%–5% peak panel reactive antibody (PRA): non-sensitized; 6%–85% peak PRA: intermediately sensitized; and >85% peak PRA: highly sensitized. P value calculated with log-rank test and corrected for multiple comparisons (Bonferroni method).

Figure 2. Minimal match criteria do not affect rejection rates for patients transplanted through the Acceptable Mismatch (AM) program. (A) The 6-month cumulative rejection incidence of highly sensitized patients transplanted through the Eurotransplant Kidney Allocation System (ETKAS) with

a minimal match level of one HLA-B and one HLA-DR antigen, or two HLA-DR antigens on the split antigen level (equivalent to minimal match criteria), or transplanted with one HLA-DR match at the broad antigen level. (B) The 6-month cumulative rejection incidence of Acceptable Mismatch (AM) patients transplanted according to the minimal match criteria of one HLA-B and one HLA-DR antigen, or two HLA-DR antigens on the split antigen level, or transplanted one HLA-DR match at the broad antigen level.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Tables

Table 1.

Parameters	Categories	ETKAS 0-5% PRA N=1991	ETKAS 6-85% PRA N=968	ETKAS >85% PRA N=121	AM N=113	Total N=3193	*P*
Sex of recipient	Female	34,3%	48,5%	59,5%	68,1%	1301	<0.001
	Male	65,7%	51,5%	40,5%	31,9%	1892	
Sex of donor	Female	48,8%	44,6%	47,9%	43,4%	1510	0.156
	Male	51,2%	55,4%	52,1%	56,6%	1683	
Age of recipient (yr)	≤50	46,3%	53,9%	64,5%	64,6%	1594	<0.001
	>50	53,7%	46,1%	35,5%	35,4%	1599	
Age of donor (yr)	≤50	57,3%	63,1%	61,2%	58,4%	1891	0.023
	>50	42,7%	36,9%	38,8%	41,6%	1302	
Donor type	HB	66,5%	73,9%	90,1%	99,1%	2260	<0.001
	NHB	33,5%	26,1%	9,9%	0,9%	933	
Repeat transplant	No	93,4%	71,6%	40,5%	46,0%	2654	<0.001
	Yes	6,6%	28,4%	59,5%	54,0%	539	
HLA-A, -B, -DR mismatch (broad antigen level)	1, 2, 3	82,5%	81,7%	84,3%	90,3%	2637	0.144
	4, 5, 6	17,5%	18,3%	15,7%	9,7%	556	
Transplant period	1996-2000	45,0%	57,5%	63,6%	42,5%	1577	<0.001
	2001-2005	55,0%	42,5%	36,4%	57,5%	1616	
Initial immunosuppression[*]	Pred / cyclo ± MMF ± IL2RA	65,8%	63,2%	64,7%	42,6%	1497	0.002
	Pred / tacro / MMF ± IL2RA	34,2%	36,8%	35,3%	57,4%	828	
Initial graft function[†]	Direct	64,5%	69,3%	67,3%	79,6%	1991	0.002
	Delayed	35,5%	30,7%	32,7%	20,4%	997	

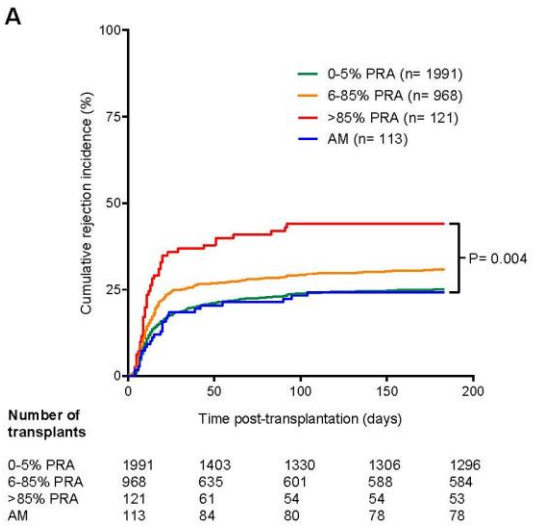
AM: acceptable mismatch, cyclo: cyclosporin, HB: heart beating, IL2RA: IL-2 receptor antagonist, MMF: mycophenolate mofetil, NHB: non-heart beating, PRA: panel reactive antibody, pred: prednisolone, tacro: tacrolimus. *Missing values (n= 868), †missing values (n= 209). *P*-values calculated with Chi Square Test.

		Cox regression								
		Univariate				Multivariate			Multivariate		
		HR	95% CI		*P*	HR	95% CI	*P*	HR	95% CI	*P*
Sex of recipient	Female (ref)										
	Male	0.797	0.489	1.300	0.364						
Sex of donor	Female (ref)										
	Male	0.949	0.599	1.504	0.824						
Age of recipient (yr)	≤50 (ref)										
	>50	0.819	0.502	1.334	0.422						
Age of donor (yr)	≤50 (ref)										
	>50	1.240	0.781	1.969	0.362						
Donor type	HB (ref)										
	NHB	1.176	0.429	4.224	0.752						
Repeat transplant	No (ref)										
	Yes	0.786	0.497	1.245	0.305						
HLA-A, -B, -DR mismatch (broad antigen level)	1, 2, 3 (ref)										
	4, 5, 6	1.353	0.712	2.570	0.356						
Luminex defined DSA	No (ref)										
	HLA class I	1.292	0.734	2.276	0.374						

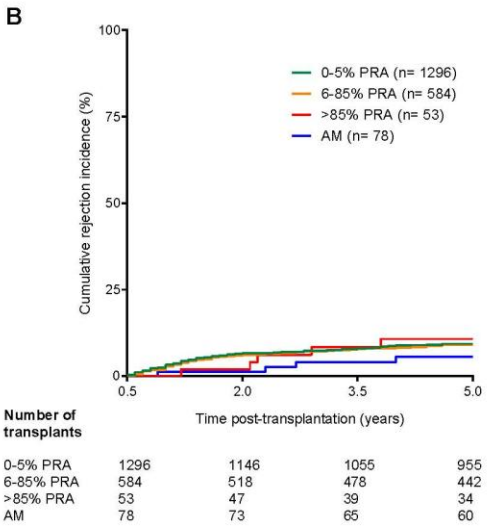
	HLA class II	0.691	0.240	1.991	0.493								
	HLA class I and class II	1.420	0.612	3.296	0.415								
Transplant period	1996-2000 (ref)												
	2001-2005	0.632	0.394	1.012	0.056					0.642	0.387	1.064	0.086
Initial immunosuppression	Pred / cyclo ± MMF ± IL2RA (ref)												
	Pred / tacro / MMF ± IL2RA	0.581	0.306	1.104	0.097	0.665	0.345	1.282	0.223				
Initial graft function	Direct (ref)												
	Delayed	1.941	1.190	3.167	0.008					1.925	1.163	3.187	0.011
Tx through AM program	No (ref)												
	Yes	0.469	0.290	0.758	0.002	0.541	0.272	1.073	0.079	0.569	0.342	0.945	0.029

Table 2. Factors affecting 6-monts cumulative rejection incidence of highly sensitized transplant recipients (>85% PRA) within PROCARE cohort (> 0 HLA-A, -B, -DR mismatch)

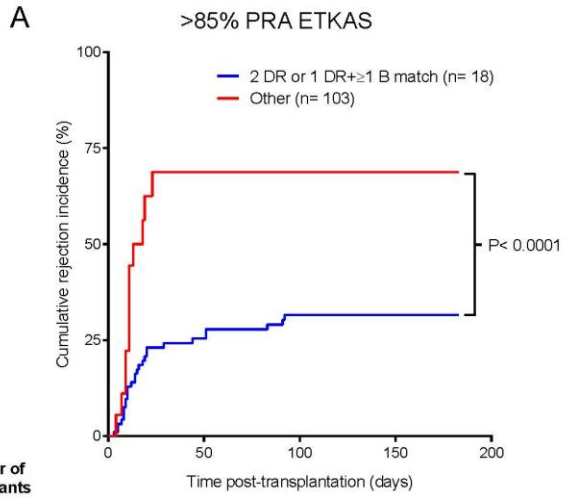
AM: acceptable mismatch, CI: confidence interval, cyclo: cyclosporin, DSA: donor specific antibody, HB: heart beating, HR: hazard ratio, IL2RA: IL-2 receptor antagonist, MMF: mycophenolate mofetil, NHB: non-heart beating, pred: prednisolone, ref: reference value, tacro: tacrolimus, Tx: transplan



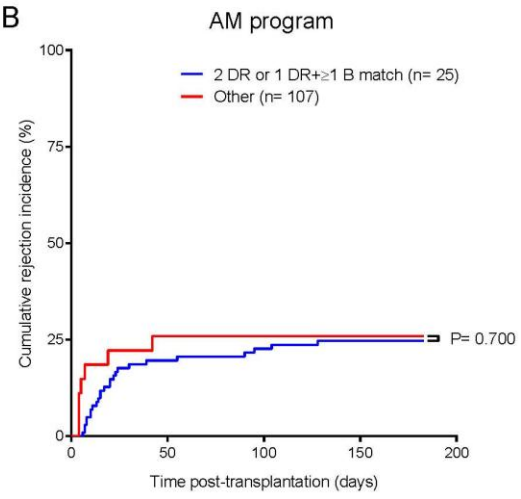
Number of transplants					
0-5% PRA	1991	1403	1330	1306	1296
6-85% PRA	968	635	601	588	584
>85% PRA	121	61	54	54	53
AM	113	84	80	78	78



Number of transplants				
0-5% PRA	1296	1146	1055	955
6-85% PRA	584	518	478	442
>85% PRA	53	47	39	34
AM	78	73	65	60



Number of transplants					
2 DR or 1 DR+≥ 1 B	18	5	4	4	4
Other	103	62	56	56	56



	25	20	20	20	20
	107	80	76	73	73